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Enantioseparations by electrochromatography with packed capillaries

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Abstract

(*S*)-Naproxen-derived and (3*R*,4*S*)-Whelk-O chiral stationary phases (CSPs) were immobilized on 3 μm silica supports and packed into 100 μm I.D. fused-silica capillaries. Enantiomers of some neutral analytes representing a variety of different classes of compounds were separated by electrochromatography using 2-(N-morpholino)ethanesulfonic acid modified with acetonitrile as the buffer system. High column efficiency (up to 200 000 plates per meter) and substantial levels of enantioselectivity were obtained in all cases with separations usually being performed in less than 10 min. © 1997 Elsevier Science B.V.

Keywords: Electrochromatography; Enantiomer separation; Chiral stationary phases

1. Introduction

Since Jorgenson and Lukacs [1] and Knox and Grant [2,3] demonstrated the potential of packed-capillary electrochromatography (CEC), this new technique has become an attractive method combining the high efficiency of capillary zone electrophoresis (CZE) with the high selectivity usually obtained in high-performance liquid chromatography (HPLC) [4,5].

Accordingly, Li and coworkers [6–8] and Lelièvre et al. [9] introduced proteins and β-cyclodextrin derivatives, immobilized on 5 μm and 7 μm silica particles, as chiral selectors for CEC. The enantiomers of several compounds were separated, some of them being of pharmacological interest, e.g. the β-adrenergic antagonist propanolol and its analogs [6–9]. Recently, imprinted polymers have also been used as chiral stationary phases (CSPs) in enantioselective CEC [10,11]. However, the aforementioned CSPs provided relatively small separation factors and limited column efficiencies. To take full advantage of the efficiency potentially available in the electrochromatographic approach, CSPs must incorporate rapid mass transfer characteristics and be capable of differentiating between enantiomers in an electrolyte (usually an aqueous medium).

We now report the chromatographic performance of two brush-type CSPs of synthetic origin in enantioselective CEC. The doubly-tethered (*S*)-naproxen-derived CSP 1 and the (3*R*,4*S*)-Whelk-O 1 CSP 2 (Fig. 1), each originally developed for HPLC

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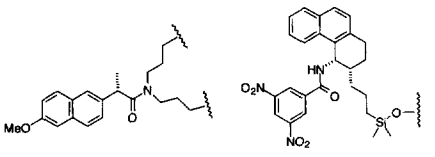

(S)-Naproxen-derived CSP 1 (3R,4S)-Whelk-O 1 CSP 2

Fig. 1. CSPs used in this study.

purposes [12,13], were bonded to silica gel (3 μm, 100 Å pore size) and packed into 100 μm I.D. capillaries. These capillaries were then used to separate the enantiomers of a variety of compounds using aqueous 2-(N-morpholino)ethanesulfonic acid modified with acetonitrile as the mobile phase.

2. Experimental

The CSPs were synthesized and immobilized on silica gel (3 μm, 100 Å pore size) as described previously [12,13]. Fused-silica was purchased from Polymicro Technologies and all capillaries (100 μm I.D.) were packed with an Anspec HPLC pump as described by van den Bosch et al. [14]. For a detection window, the polyimide coating was burned off as close as possible downstream from the second frit.

Electrochromatography was performed using an HP ^{3D}CE system equipped with an autosampler, a Chemstation and a diode-array UV detector. The HP ^{3D}CE system allows application of a pressure up to of 10 bar to the inlet and/or outlet vial. After packing, the capillaries were flushed with the buffer system for 2 h and stabilized with pressure on both vials by slowly and smoothly increasing the applied voltage to 25 kV. All runs were performed with a pressure of 10 bar on both vials to prevent bubble formation in the capillary. The oven temperature was set at 25°C. On-column detection was carried out by UV absorbance at 230 nm and 254 nm. Samples were injected electrokinetically by applying a voltage of 5 kV for 3 s. Changing the injection time did not affect peak width and column efficiency.

The 2-(N-morpholino)ethanesulfonic acid (MES) and tris(hydroxymethyl)aminomethane (TRIZMA) were purchased from Sigma. The buffer solutions were first titrated with sodium hydroxide or hydrochloric acid (Fisher Scientific) to pH 6.0 and pH 8.0 at a concentration of 25 mmol, respectively, and then mixed with 3.5 volumes of acetonitrile (EM Science). All test analytes were available from prior studies and were dissolved in MES buffer–acetonitrile (1:3) (1–2 mg/ml). The slight mismatch in the injection–mobile phase composition causes a baseline perturbation that was used as the marker of the electroosmotic flow (EOF). All solutions were made with deionized water obtained from a Milli-Q^{50} system. The running buffer and the sample solutions were degassed in an ultrasonic bath for 5 min.

3. Results and discussion

Using the procedure for packing and frit fabrication described by van den Bosch et al. [14], we were able to pack capillaries providing high chromatographic performance and stability in enantioselective CEC. In contrast to the claim made by Li and Lloyd [6] that one can not make frits directly from Pirkle-type CSPs, we find that satisfactory frits can be prepared directly by sintering CSP 1 and CSP 2.

Because of our experience using these CSPs for reversed-phase HPLC, acetonitrile was initially chosen as the modifier because it usually results in less retention of the analytes than does a comparable concentration of methanol. Additionally, a comparison with various alcoholic modifiers demonstrated that the use of acetonitrile affords the higher EOF of the buffer system [6]. In general, flushing the freshly-packed capillaries with the running buffer for 2 h followed by stabilization for 1 h provided stable and reproducible conditions. However, stable conditions were not obtained when working with TRIZMA (25 mmol, pH 8.0) modified with acetonitrile (1:3.5, v/v) even after flushing and conditioning for several days. As was suggested by Lelièvre et al. [9], we used baseline perturbations as the marker of the EOF. These perturbations are easily observed with sample solutions containing a slightly lower concentration of the organic modifier than the running buffer system. The pressure of 10 bar on the inlet and outlet buffer vials during the run prevent bubble formation even at currents greater than 8 μA.

Test analytes 1–10, representing a variety of compound classes, were used to study the usefulness

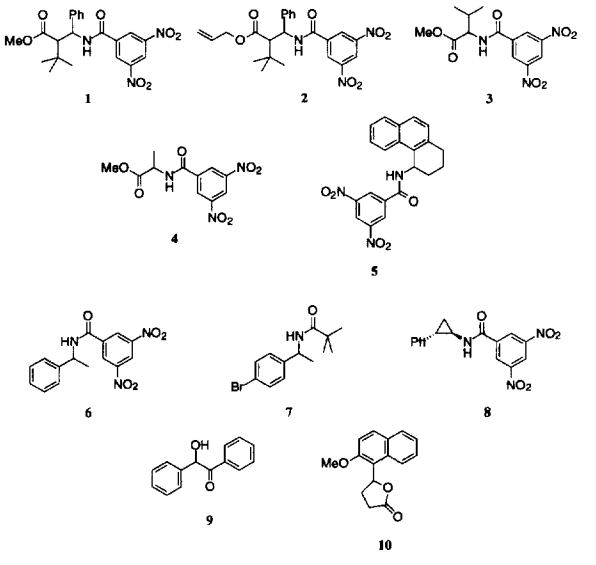

Fig. 2. Structure of the test analytes.

of these brush-type CSPs under reversed-phase conditions in CEC (Fig. 2). The enantiomers of these compounds are baseline-separated using MES (25 mmol, pH 6.0) diluted with 3.5 volumes (v/v) of acetonitrile as the mobile phase, as is shown for 3 and 7 (Figs. 3 and 4). All ten analytes show high enantioselectivity and column efficiency on both CSPs (Tables 1 and 2).

For enantiomer separations one expects the more retained enantiomer to show lower chromatographic efficiency than its antipode (i.e. $N_1 > N_2$). In this regard, CSP 2 shows relatively less efficiency loss than CSP 1 and, in the case of benzoin, 9, N_2 is greater than N_1 (Table 2).

For the enantioseparation of 7 on CSP 2, a separation factor, α, of 3.82 and 200 000 theoretical plates per meter were observed for the less retained enantiomer. These data show that both CSP 1 and CSP 2 realize the chromatographic efficiency inherent in the CEC packed capillary approach. This is in contrast to earlier reports where protein-derived, cyclodextrin-derived, or imprinted polymeric CSPs did not provide both high selectivity and high efficiency. The quality of the chromatographic performance of these brush-type CSPs is attributed to the use of smaller particle packings, i.e., 3 μm silica instead of 5 μm, and to the favorable mass-transfer

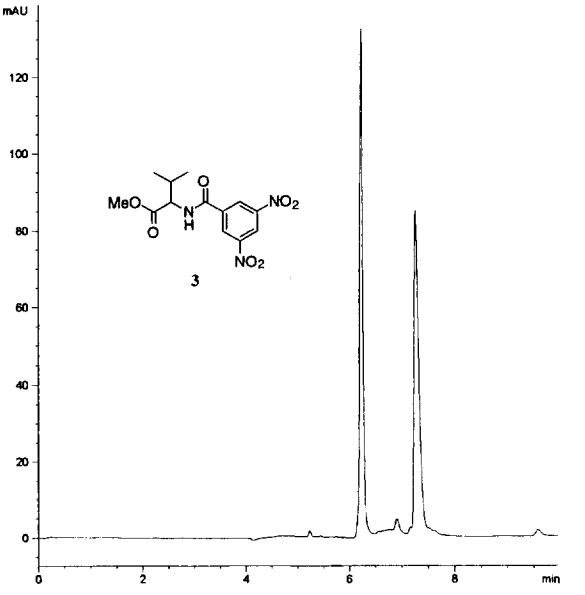

Fig. 3. CEC separation of the enatiomers of 3 on CSP 1. Conditions: MES (25 mmol, pH 6.0)–acetonitrile (1:3.5); 25 kV, 38.0 total capillary length; 29.5 cm effective length; UV detection at 230 nm.

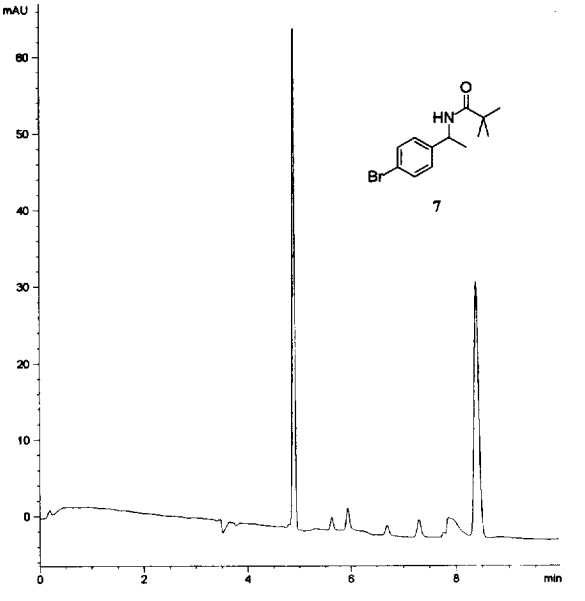

Fig. 4. CEC separation of the enatiomers of 7 on CSP 2. Conditions: MES (25 mmol, pH 6.0)–acetonitrile (1:3.5); 25 kV, 39.3 total capillary length; 30.5 cm effective length; UV detection at 230 nm.

Table 1
CEC enantioseparations of 1–5 on CSP 1

Compound	k_1	α	$^{N}_{1}$/m	N_2/m	R_s
1	0.77	1.90	195 000	68 000	13.47
2	0.86	1.78	173 000	49 000	11.30
3	0.53	1.49	196 000	114 000	8.02
4	0.51	1.33	170 000	121 000	5.61
5	1.28	2.80	121 000	24 000	16.86

Conditions: MES (25 mmol, pH 6.0)–acetonitrile (1:3.5); 25 kV, 38.0 total capillary length, 29.5 cm effective length.

Table 2
CEC enantioseparations of 6–10 on CSP 2

Compound	k_1	α	$^{N}_{1}$/m	N_2/m	R_s
6	0.61	1.59	180 000	154 000	11.48
7	0.34	3.82	200 000	160 000	30.95
8	0.75	1.29	157 000	145 000	6.29
9	0.26	1.23	176 000	189 000	2.63
10	0.54	1.54	182 000	170 000	10.14

Conditions: MES (25 mmol, pH 6.0)–acetonitrile (1:3.5); 25 kV, 39.3 total capillary length, 30.5 cm effective length.

kinetics of these CSPs. Similar comments were made previously for CSP 2 in both HPLC and supercritical fluid chromatography applications [15,16].

One might suppose that a doubly-tethered CSP would provide a better coverage of silanol groups than does a singly-tethered selector and thus afford a smaller zeta potential on the surface of the packing. In accord with this view, the electroosmotic mobility, μ_{eof}, of CSP 1 was determined to $1.83 \cdot 10^{-4}$ cm^2/V s and $2.24 \cdot 10^{-4}$ cm^2/V s for CSP 2. As is well known from CZE, high reproducibility requires control of the EOF. The relative standard deviations (R.S.D.s) of the electroosmotic mobility calculated from all runs and the R.S.D.s of the retention factor of the less retained enantiomer, k_1, and the separation factor of the enantiomers of 4, α, on CSP 1 are shown in Table 3. The data demonstrate the consistently high run-to-run, day-to-day and column-to-column reproducibility observed for all enantioseparations on both CSPs.

Table 3
Reproducibility of electroosmotic mobility, retention factor and separation factor

	Run-to-run reproducibility R.S.D. (%)	Day-to-day reproducibility R.S.D. (%)	Column-to-column reproducibility R.S.D. (%)
μ_{eof}	0.51	1.32	0.66
k_1	3.46	3.19	2.24
α	0.53	1.19	0.76

Relative standard deviations (R.S.D.s, %) of retention factor and separation factor were calculated from the enantioseparations of 4. All data were obtained with CSP 1 at 25 kV using MES (25 mmol, pH 6.0)–acetonitrile (1:3.5) as the mobile phase.

4. Conclusions

It has been shown that enantioselective analysis done by packed-column CEC can afford both high selectivity and high levels of chromatographic efficiency. CSP 1 and the CSP 2 were each packed into capillaries and frits were prepared by sintering these packing materials directly. Using MES buffer modified with acetonitrile, the enantiomers of all ten test analytes were separated reproducibly with resolution values ranging from 2.6 to 31.

As a result of favorable mass-transfer kinetics, these brush-type CSPs offer high chromatographic performance and short analysis time in CEC. Most enantioseparations were found to require less than 10 min.

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References

[1] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209.
[2] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[3] J.H. Knox, I.H. Grant, Chromatographia 32 (1991) 317.
[4] A.L. Crego, A. González, M.L. Marina, Crit. Rev. Anal. Chem. 26 (1996) 261.
[5] M.M. Dittmann, G.P. Rozing, J. Chromatogr. A 744 (1996) 63.
[6] S. Li, D.K. Lloyd, Anal. Chem. 65 (1993) 3684.

[7] S. Li, D.K. Lloyd, J. Chromatogr. A 666 (1994) 321.
[8] D.K. Lloyd, S. Li, P. Ryan, J. Chromatogr. A 694 (1995) 285.
[9] F. Lelièvre, C. Yan, R.N. Zare, P. Gareil, J. Chromatogr. A 723 (1996) 145.
[10] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, J. Liq. Chromatogr. 20 (1997) 1489.
[11] L. Schweitz, L.I. Andersson, S. Nilsson, Anal. Chem. 69 (1997) 1179.
[12] W.H. Pirkle, C.J. Welch, B. Lamm, J. Org. Chem. 57 (1992) 3854.
[13] W.H. Pirkle, P.L. Spence, B. Lamm, C.J. Welch, J. Chromatogr. A 659 (1994) 69.
[14] S.E. van den Bosch, S. Heemstra, J.C. Kraak, H. Poppe, J. Chromatogr. A 755 (1996) 165.
[15] C. Villani, W.H. Pirkle, Tetrahedron Asymm. 6 (1995) 27.
[16] C. Wolf and W.H. Pirkle, J. Chromatogr. A, (1997) in press.